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Methylene Blue Mediated Laser Therapy of Maxillary Sinusitis

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Abstract—The purpose of the present work is a clinical study of photodynamic therapy of maxillary sinusitis. 0.1%-Methylene Blue aqueous solution in combination with He–Ne laser irradiation (632.8 nm) have been used for treatment of maxillary sinus mucous of patients with acute and chronic maxillary sinusitis. The differences between the results of the treatment with dye and light versus treatment with a drug for every group of patients were statistically analyzed by Student's *t* test. The efficacy of the photodynamic therapy was estimated with the use of the following criteria: the state of respiration, olfaction, duration of purulent discharge, reconstruction of transport function of ciliary epithelium, etc. The obtained results have shown that the photodynamic therapy is effective in comparison with conservative methods of treatment of the disease.

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1. INTRODUCTION

Photodynamic therapy (PDT) is a promising method in modern medicine. Whereas PDT use in cancer treatment has been widely accepted [1–4], antimicrobial PDT is not a well-developed technology [5], although it is successfully used for treatment of various inflammatory diseases [5–13]. The problem of treatment of maxillary sinusitis remains important in rhinology despite wide application of novel pharmaceutical and surgical methods of treatment of the disease [14–17]. However, application of photodynamic therapy for treatment of rhinological diseases has not yet been studied in detail [18].

Development of novel photodynamic treatment technologies includes knowledge of photosensitizer diffusion rates, the proper choice of a laser source and optimal conditions for the laser irradiation, and delivering the photosensitizers into human target tissue. Methylene Blue (MB) and a He–Ne laser are very appropriate as a photosensitizer and a light source due to the high efficiency of light utilization followed by MB molecule excitation, their biocompatibility, and low cost. There are numerous investigations related to the application of MB in photodynamic therapy [8, 9, 14–17]. However, the use of MB-laser therapy for the treatment of patients with maxillary sinusitis has not yet been presented in literature.

In this study, for the first time we present the clinical results of administration of MB in combination with He–Ne laser irradiation (632.8 nm) for photodynamic therapy of patients with maxillary sinusitis.

2. STRUCTURE AND OPTICAL PROPERTIES OF MUCOUS MEMBRANE

The mucous membrane plays a leading role in the physiology of the nose and paranasal sinuses [19, 20]. It is covered with a pseudostratified epithelium. The membrane called basic divides epithelial and proper layer of the mucous tissue, which is similar in structure to connective tissue.

In the proper layer, venous plexuses, which consist of an upper network of smaller vessels and a deeper network of larger vessels, are arranged. Normally, the total thickness of the mucous membrane varies from 0.1 to 0.5 mm [19, 20]. In the presence of pathology (maxillary sinusitis, rhinitis, or other rhinological disease), the thickness of the mucous membrane considerably increases and can reach 2–3 mm [19, 21].

The optical properties of the mucous membranes are mainly determined by the optical properties of the lamina propria mucosae (LPM) layer of fibrous connective tissue underlying the epithelial layer, since this layer is much thicker than the epithelial layer. The scattering of the tissue both in the visible and NIR is defined by the main scatterers: collagen and elastin fibers [22].

In the visible range of wavelengths, the spectrum of absorption of the mucous is defined by absorption bands of oxyhemoglobin (415, 540, and 575 nm) of blood localized in a venous plexuses of the LPM. In the NIR spectral range, the form of the absorption spectrum is defined by absorption bands of water (986, 1199, 1453, and 1936). Pathologically changed mucous membrane is characterized by a high degree of swelling and blood-fullness that is reflected in the form of strongly pronounced absorption bands of oxyhemoglobin and water in the absorption spectrum of the mucous membrane [22]. In the spectral range from 600 to 700 nm, which corresponds to absorption bands of MB (608 and 662 nm), the intrinsic absorbance of the mucous tissue is low (see, Fig. 1), which can provide a high selectivity of the photodynamic action of the dye.

3. MATERIALS AND METHODS

3.1. Patient Selection

One hundred forty patients of both sexes (aged from 20 to 41 years) with rhinosinusitis were enrolled for the study. Sixty-five patients were diagnosed as having acute maxillary sinusitis (AMS) and 75 patients were diagnosed as having an acute condition of chronic maxillary sinusitis (CMS). Diagnosis of all patients was confirmed by X-ray imaging. Separation of the patients was carried out in accordance with the classification of Piskunov [20].

Patients with AMS were divided into two groups depending on the treatment protocol: in group I, 30 patients were treated with photodynamic therapy; in group II (the control group), 35 patients were treated by traditional (pharmaceutical) therapy.

Patients with CMS were also divided into two groups: in group I, 35 patients were treated with photodynamic therapy; in group II (the control group), 40 patients were treated by traditional (pharmaceutical) therapy.

Patients were excluded if they had had surgery treatment of maxillary sinuses before the photodynamic or pharmaceutical therapy. All patients gave their informed consent for participation in the study.

3.2. Microbiological Examination

Bacteriological examination was carried out to define the etiology of the pathological process in maxillary sinus. During the topical anesthesia with 10% lidocaine solution, a puncture of the maxillary sinus was made with the standard method and discharge sampling was taken by sterile syringe. The samples were put into a test tube with meat infusion broth. The obtained material was diluted 1 : 9 and inoculated in Petri dishes. As a nutrient medium at the bacterial inoculation, 5% blood agar medium was used. The samples were put into a thermostat with a temperature of 37°C for 24 h. Microbiological examinations were taken before and after the treatment.

3.3. Pharmaceutical Therapy Protocol

The control groups took traditional therapy including antibiotic therapy—intramuscular injections of lincomycin (0.6–0.9 g/day) or cephazolin (1–2 g/day). To obtain a high concentration of drugs in the nidus of inflammation, lavage of maxillary sinuses with solutions of antibacterial drugs and physiotherapeutic methods of treatment were carried out. The course of treatment was 5–7 days for the patients with AMS and 12–13 days for the patients with CMS.

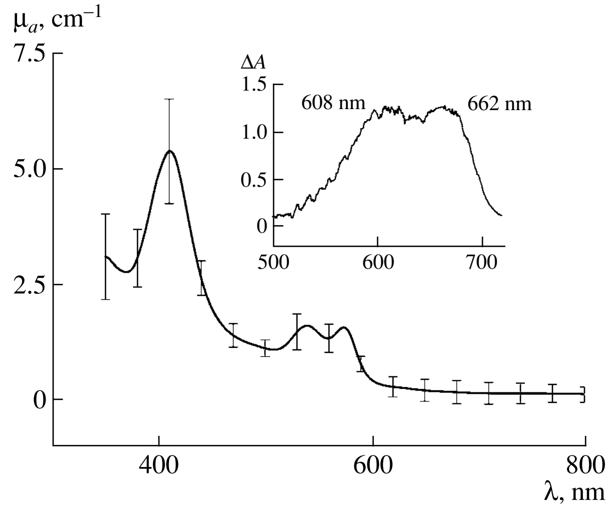

Fig. 1. The wavelength dependence of the absorption coefficient μ_a of human mucous tissue calculated using the inverse adding-doubling method [22]. The solid line corresponds to the averaged experimental data and the vertical lines show the standard deviation values. Inset shows absorbance spectrum of mucosa 30 min after start of staining by methylene blue solution.

3.4. Light Source

For photodynamic treatment of maxillary sinusitis, the AFL-1 laser system on the basis of a GN-80 He–Ne laser (λ = 632.8 nm) (Russia) was used. Laser light was delivered to the target tissue via an optical fiber with a diameter of 0.9 mm and a core diameter of 400 μm. To provide homogeneous irradiation of the maxillary sinus surface, an original fiber-optic diffuser was designed. The power of the laser system on the fiber tip was 40 mW. Since the area of one maxillary sinus varied from 14.6 to 29.2 cm^2, the power density on the tissue surface varied from 1.4 to 2.7 mW/cm^2 within the sinus.

3.5. PDT Protocol for Treatment of Sinusitis

For PDT, the following protocol was used. Before the treatment, a puncture of the maxillary sinus through the nasal duct was made with a standard aspirating needle. Then, a catheter with a diameter of 1 mm was injected through the needle. The lavage of the maxillary sinus of a patient was performed by physiological solution with the use of the catheter, after which 1.5 ml of the aqueous 0.1% MB solution (Sigma, United States) was administered into the maxillary sinus. For the patients with CMS, persistent drainage was carried out for 10 days during the treatment. The staining time was 30 min. Before irradiation, the lavage of the maxillary sinus of the patient by physiological solution was again performed.

Frequency of discovery of microflora at both acute and chronic maxillary sinusitis

Forms of microflora	Frequency of discovery	
	acute maxillary sinusitis	acute condition of chronic maxillary sinusitis
S. aureus	21.5	22.6
S. pneumoniae	18.5	20.1
S. epidermidis	16.9	16
S. viridans	10.8	12
S. pyogeneus	9.2	9.3
Pr. vulgaris	–	9.3
S. heaemoliticus	3.0	–
E. coli	1.6	–
Lack of growth	18.5	10.7
Total	100	100

Laser radiation was delivered into the maxillary sinus by optical fiber with the fiber-optic diffuser. The exposition time was 15 min per treatment. The dose of one irradiation was 1.3–2.4 J/cm^2. The course of the treatment was 1–5 procedures for the patients with AMS and 3–8 procedures for the patients with CMS. The total dose of the irradiation was 1.3–19.2 J/cm^2 depending on the number of the PDT treatments.

3.6. Testing of Recovery

The differences between the results of the treatment with dye and light versus treatment with a drug for every group of patients were statistically analyzed by Student's *t* test. A *p* value of less than 0.05 was taken to indicate statistical significance. Efficacy of the PDT was estimated with the use of the following criteria: the state of nasal breathing, olfaction, duration of purulent discharge, reconstruction of transport function of ciliary epithelium, and hospital stay. An estimate of global blood analysis was made from the index of the erythrocyte sedimentation rate and the change in the leukogram.

4. RESULTS

4.1. Microbiological Analysis

During microbiological examination, different pathogens were detected. Data of the microbiological analysis for the patients with AMS and CMS are presented in the table. It is seen that the main bacterial strains that accompanied the sinusitis development were *Staphylococcus aureus* (21.5% for AMS and 22.6% CMS), *Streptococcus pneumoniae* (18.5% for AMS and 20.1% for CMS), and *Staphylicoccus epidermidis* (16.9% for AMS and 16% for CMS). Lack of bacterial growth was demonstrated for 18.5 and 10.7% of smears from maxillary sinuses of AMS patients and CMS patients, respectively. In all groups, lack of bacterial growth was demonstrated after the PDT treatment.

4.2. Treatment of Patients with Both Acute and Chronic Maxillary Sinusitis

Signs of clinical recovery of the patients were defined in accordance with data of objective investigations. During the rhinoscopy, a reduction in swelling and hyperemia of a mucous membrane and a decrease in the periosteal reaction at the palpation in the region of nasal cavities were observed. Restoration of olfaction and transport function of the ciliary epithelium was registered. In addition to X-ray data about the state of nasal cavities, the status of peripheral blood, normalization of body temperature, and duration of stay of patients in hospital were considered.

The results of the observations of the patients with AMS from both PDT and control groups during the treatments show that the recovery of all clinical signs took place faster for the first group (PDT) than for the second group (traditional therapy). Comparison of the values of the clinical signs using Student's *t* test shows that the differences in the data between the two groups of patients were statistically significant ($p < 0.05$). All data are presented in Fig. 2. Vertical lines show the standard deviation values.

All patients with acute conditions of CMS required more prolonged course of the PDT treatment for relief of symptoms of the disease than the patients with AMS. However, statistical analysis has shown that the differences in the data between the control and the PDT group of patients were statistically significant ($p <$ 0.05). Data with the signs of clinical recovery of the patients are presented in Fig. 3. Vertical lines show the standard deviation values.

The results of the treatment were evaluated during the total course of the therapy and in a prolonged period (two years) after that. They have demonstrated the beneficial effect of PDT on the inflammation process; positive dynamics was observed in 100% of cases for patients with AMS.

When applying PDT for treatment of patients with CMS, full recovery was observed for 54.3% of patients. In the control group, full recovery was observed for 50% of patients. Satisfactory results (insignificant difficulty of respiration, etc.) were observed for 31.4% of patients from the first group. In the second group, the satisfactory results were observed for 27.5% patients. Nonsatisfactory results were obtained for 14.3% patients from the first group and for 22.5% patients from the second group. In the cases the patient complaints were difficulty of nasal respiration and purulent discharge. Such patients had been subjected to the standard operative treatment of maxillary sinus disease [23]. The observations during the two years after the treatment have shown the efficacy of PDT for treatment of patients with chronic maxillary sinusitis. Positive

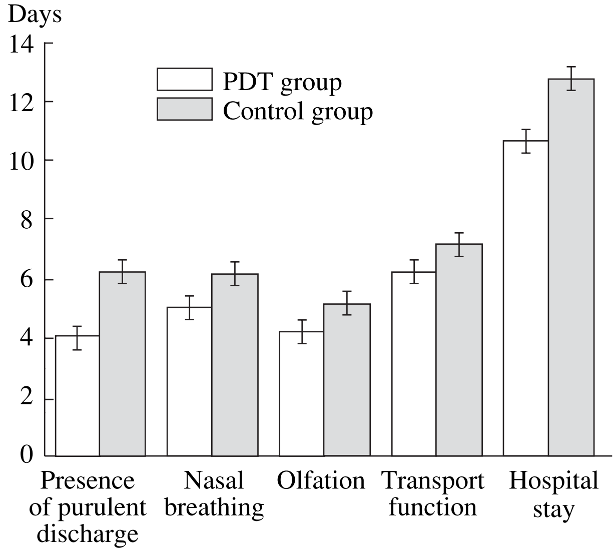

Fig. 2. Diagram of clinical recovery signs of patients with acute maxillary sinusitis. Light gray and dark gray columns correspond to the group of patients treated by the PDT method and control group, respectively. Vertical lines show the standard deviation values.

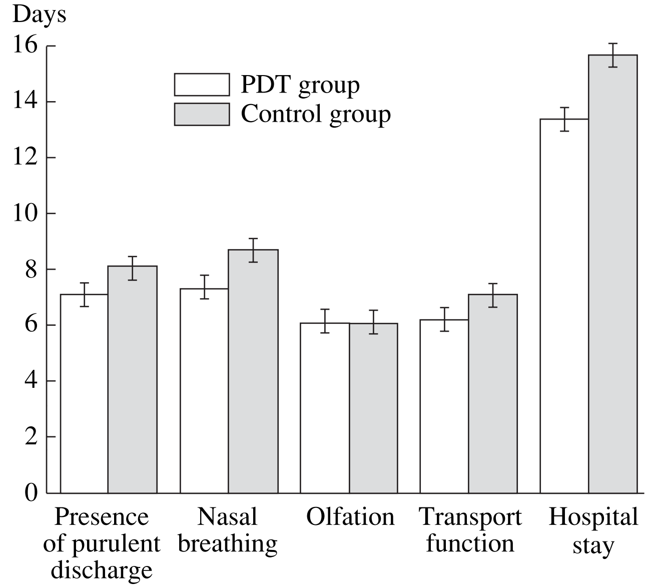

Fig. 3. Diagram of clinical recovery signs of patients with chronic maxillary sinusitis. Light gray and dark gray columns correspond to the group of patients treated by the PDT method and control group, respectively. Vertical lines show the standard deviation values.

dynamics was observed in 85.7% of cases. For the patients treated with traditional therapy, this was the case in only 77.5% of cases.

In the study of peripheral blood of patients before and after the treatments, significant differences in the indices of peripheral blood between both groups were not observed. This shows that PDT did not influence the hematopoietic function and did not have a sensitizing action on the organism.

Observations over two years on the 37 patients with AMS have shown that, in the PDT group, relapses took place in 11.1% of cases, and in the control group there relapses in 26.3% of cases. Observations of the 44 patients with CMS show that, in the PDT group, relapses took place in 55.5% of cases and in the control group in 82.3% of cases. It is worth noting that the frequency of the relapses of maxillary sinusitis in the compared groups differed. The patients treated with traditional therapy had relapses 1–2 times a year. The frequency of relapses of patients after PDT was once a year or once in two years.

5. DISCUSSION AND CONCLUSIONS

According to our findings (see table) and those in the literature [24–27], the most prevalent bacteria found in nasal discharge of patients with AMS and CMS are gram-positive *Staphylococcus aureus* (*S. aureus*) and *Streptococcus pneumoniae* (*S. pneumoniae*). The observed lack of bacterial growth for some of the patients can be explained either by previous antibiotic therapy or viral etiology of the disease or by the presence of anaerobic flora, a special examination of which we did not carry out.

It was shown that MB with red light has a high photobactericidal activity against *S. aureus* [5, 6, 9, 28, 29], *S. epidermidis* [5, 6], *S. pyogenes* [5, 6], *S. pneumoniae* [30], and other pathogens. However, among gram-positive bacteria, the correlation between the dye photobactericidal efficacy and dimerization was observed [30]. It was found that a number of bacteria killed with MB and light at the optimal dimer wavelength of excitation rose faster then those killed at the optimal monomer wavelength of excitation with increasing dye concentration. These results demonstrated the involvement of the dye dimers in bacterial killing.

The spectrum of the dye represented a superposition of monomer and dimer bands with a different contribution of the latter one. The monomer and dimer bands are located at 662–664 nm and at 605–610 nm, respectively (see Fig. 1). A third band, which would correspond to higher dye aggregates and is located in the region 560–580 nm, was not observed [30]. It is well known that the MB dimer concentration increases with increasing dye concentration. In addition, the electrostatic interaction of cationic dye with negatively charged polymers on the cell surface induces dimerization of adjacent dye molecules bound to the anionic site of polymers and the formation of bound dimers on the cell's external surface (including bacterial cells). It was shown that bacteria induce additional dimerization of MB [30].

During the staining, gradual dimerization of the dye in mucous was observed. As was shown earlier [31] in one-side diffusion of MB solution in pathologically changed mucous samples in vitro, the average time of total staining of tissue was about 30–40 min. During that time, the height of the peak corresponding to MB dimer absorption (608 nm) became equal to or higher than that of the monomer (662 nm). Thus, 30 min is enough to obtain maximal staining of the mucosa and dimerization of the dye in tissue.

Irradiation of the tissue at a wavelength of 632.8 nm is effective because the wavelength is within the absorption band of MB dimer. It is important that the administration of MB be topical, because systemic administration results in the reduction of the dye to the colorless leukoform [32].

After light absorption by the MB molecule, intersystem crossing results in the occupation of the metastable triplet level. This creates the possibility for energy transfer to molecular oxygen, which is in the ground triplet state. The singlet oxygen produced by the reaction induces cytotoxic reactions [33–35].

Molecules of proteins and lipids forming the cell membranes are easy to photooxidize. This process leads to the loss or change of the functions of photodamaged molecules, which causes morphological changes in the cell. Thus, MB sensitizes damage of the membrane under the action of He–Ne laser radiation in the place of MB molecule localization. The damage of the membrane causes the liberation of the photosensitizer from the place of its localization, the redistribution around the cell, and the damage of secondary targets such as mitochondria and the Golgi complex [35, 36].

After treatment, the lack of bacterial growth in all groups of the patients shows that PDT has a pronounced antibacterial effect. In [5, 6, 9, 30], it was reported that laser irradiation of power densities 5–100 mW/cm^2 and doses 5–24 J/cm^2 should be provided for effective photodynamic killing of pathogens. Fractionated irradiation with dose accumulation enhances the photodynamic effect [37]. In many studies it was shown that, for many reasons, the resistance of microbes to photodynamic action is considerably lower than that of surrounding tissues. Among these reasons are the following: fast microbial uptake of dye, small dimensions (more than one order less than tissue cells), and the specificity of wall structure. These may lead to more effective targeting followed by damage to the microorganism by light [6, 38]. We used a low power density and maximal dose of 2.4 J/cm^2 for a single irradiation to exclude tissue cell damage. The course of the treatment was 1–8 procedures, and the maximal total dose of irradiation did not exceed 19.2 J/cm^2. Thus, the fragmentariness of delivered laser irradiation provided a dosing regime whereby bacteria could be killed effectively without damaging adjacent mucous cells. In addition, such a regime of the treatment allows the irradiation dose to be varied for maximal effectiveness and safety of the procedure for each patient.

Soft photodynamic action can cause pathological epithelial cell apoptosis (instead of necrosis observed in cases of intensive photodynamic action [1–4]), induced cell phagocytosis, pathological cell removal, and normal tissue restoration; thus, there is less opportunity for microorganisms to grow and penetrate inside tissue [39].

It should be noted that, in addition to the antibacterial effect connected with the action of exogenous dye MB, low-intensity laser irradiation in the red range of the spectrum may have its own biological action on the cells [39]. The primary acceptors of the irradiation may be endogenous porphyrins and other endogenous chromophores, which are photosensitizers for the generation of singlet oxygen and other radicals. As known, the porphyrin content in an organism is increased at many pathological states of a human. Since at an acute condition of maxillary sinusitis the mucous membrane becomes swelled and blood-filled, lymphocytes, leucocytes, lipoproteins, and other components can be the targets for laser irradiation. Interaction of porphyrins and other endogenous chromophores with low-intensity laser irradiation in the range around 633 nm may activate reactions causing an improvement in blood microcirculation, tissue regeneration, and immunomodulating activity [39]. It was also shown experimentally that red light (633 nm) at very low power densities and doses of about 2 J/cm^2 can regulate (increase) the concentration of intracellular free calcium Ca^{2+} of macrophages and therefore regulate their immunocompetence [40]. The superoxide (SO) and nitric oxide (NO) are produced by activated macrophages and may be responsible for host defense against microorganisms [41]. Hepatocyte experiments have shown that He–Ne laser radiation at 633 nm at a dose of 0.24 J/cm^2 and power density of 12 mW/cm^2, either directly or in a cascade-like effect depending on the increase in cell Ca^{2+}, can cause cell stimulation [42].

For patients who were given traditional treatment with drugs, both topical inflammation and intoxication of the organism were more prolonged. This is seen from the duration of the hospital stay of the patients from the groups under investigation. It is related to the injurious effect of high doses of topically and systemically applied antibiotics with wide action spectrum on the immune reaction of the organism and initiation of bacterial resistance.

Thus, the clinical study of photodynamic therapy with the use of MB and radiation of a He–Ne laser on patients with both acute maxillary sinusitis and an acute condition of chronic maxillary sinusitis showed the pronounced clinical efficacy of the method, which is more beneficial than traditional antibacterial treatment.

In conclusion, it is worth noting that, in recent years, the development of the LED has advanced to a stage where their use in phototherapy is possible. An LED offers several advantages for clinical use. The choice of emission wavelength ranges from UVA to near infrared. The bandwidth is 5–10 nm. In addition, LEDs are inexpensive (in comparison with all other light sources). Moreover, they can be arranged in different geometric combinations to compensate for difficult anatomic areas, in particular, in the sinus [43]. The results of clinical and laboratory studies presented in [44–46] show the high effectiveness of antibacterial therapy based on the photodynamic effect caused by the action of red LED light (660 nm) and MB (0.1%) on pathogenic microorganisms, at light doses on a level of 1–16 J/cm^2. Clinical results have a good correlation with results of bacterial research conducted in vivo and in vitro. Infrared irradiation (810 nm) and indocyanine green (ICG) dye is also an effective combination for killing microbes [45]. This means that soft PDT using such dyes as MB and ICG and low-intensity light sources may be considered as a realistic treatment option, and it is likely that the interest in this therapeutic modality will increase.

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